AD_____

Award Number: W81XWH-10-2-0171

TITLE: Minocycline and N-acetylcysteine: A Synergistic Drug Combination to Treat Traumatic Brain Injury

PRINCIPAL INVESTIGATOR: Peter Bergold, Ph.D.

CONTRACTING ORGANIZATION: State University of New York-Downstate Medical Center
Brooklyn, NY 11203

REPORT DATE: October 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October	Annual	30 September 2010 – 29 September 2011

4. TITLE AND SUBTITLE

Minocycline and N-acetylcysteine: A Synergistic Drug Combination to Treat Traumatic Brain Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0171

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Peter Bergold, Ph.D.

E-Mail: pbergold@gmail.com

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

State University of New York-Downstate Medical Center
Brooklyn, NY 11203

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Background: Due to the changing nature of war, there is a pressing need for new treatments for traumatic brain injury (TBI). The grantee previously found screened that the combination of minocycline (MINO) and N-acetyl cysteine (NAC) synergistically improved brain function when dosed one hour following closed cortical impact (CCI) in rats. The overall objective of this proposal is that MINO/NAC synergistically improves brain function following TBI. Four tasks will be done to achieve this objective: 1) Differing doses of MINO/NAC will be tested for the ability to improve behavior and histology following moderate CCI. 2) MINO/NAC was effective when dosed one hour after injury. Longer intervals between injury and drug dosing will be tested. 3) TBI is clinically heterogenous; MINO/NAC should restore brain function following an additional TBI model, lateral fluid percussion. 4) A restoration of cognitive function will be tested three months after CCI. The grantee has received both institutional and DoD approval for the animal experiments in this work and has trained a MD-Ph.D. candidate to perform these studies.

15. SUBJECT TERMS
behavioral restoration, therapeutic window, FDA-approved drugs, isobolistic analysis, animal TBI models, long-term efficacy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

There is a pressing need for new treatments for TBI because of the increased frequency of head injuries during Operation Iraqi Freedom and Operation Enduring Freedom. Multidrug treatment is one of many therapeutic approaches that are being tested. The applicant previously performed a screen of drug pairs to treat TBI ("Multidrug treatment of traumatic brain injury", PT073028) from the Fiscal Year 2007 CDMRP program for Psychological Health/Traumatic Brain Injury. Drug pairs were screened that: (1) limited TBI as monotherapy in preclinical or early clinical trials and (2) have FDA approval for uses other than TBI. The drug screen used a novel behavioral assessment with high cognitive demand to find drug combinations that best restored brain function following moderate or mild closed cortical impact (CCI) (1). The applicant found that MINO and NAC improved both cognitive and histological outcomes following CCI (2). As monotherapy, MINO-treated rats learned, but had no 24-hour retention of an active avoidance task. NAC-treated rats were greatly impaired in both acquisition and retention. The combination of MINO plus NAC, given one hour after injury produced a large improvement in both acquisition and retention. NAC alone had no effect indicating a synergistic drug interaction with MINO in improving cognitive function. In addition, MINO plus NAC-treated rats had better sparing of both white and grey matter. These observations provide the justification for the central hypothesis that: MINO plus NAC synergistically improve brain function following TBI. MINO plus NAC has the potential to rapidly get a safe and effective combination therapy into clinical trials. The combination is likely to be safe. Both drugs have been used in the clinic for decades with well-known pharmacokinetics, pharmacodynamics and drug interactions. The current project contains key experiments needed to begin testing MINO plus NAC in the clinic.

Body

The statement of work for this project describes four tasks:

Task 1. Optimizing the dosing of MINO plus NAC in the CCI model of TBI.

Task 2.Evaluation of the Therapeutic Window of MINO plus NAC

Task 3. Determine the efficacy of MINO plus NAC in the lateral fluid percussion model of TBI

Task 4. Examination whether MINO plus NAC provide long-term behavior improvements.

Regulatory approval has been obtained for the animal work in this project by the SUNY-Downstate Institutional Animal Care and Use Committee as well as the USAMRMC Animal Care and Use Review Office. These approvals cover all four tasks in the statement of work.

In October, 2011 one key member of my research team, Samah Abdel-Baki, M.D. left the laboratory on short notice. Dr. Abdel-Baki was the first author on two previous studies of Dr. Bergold was recruited into a more lucrative position running a clinical research project (1, 2). In July 2011, Dr. Natalia Grin'kina, a post-doctoral fellow, and Ms. Margalit Haber, a Ph.D. graduate student with Dr. Bergold.

Dr. Grin'kina and Ms. Haber are in the process of becoming skilled in the the CCI model. To test their ability to perform the CCI surgery, rats were divided into two groups. In sham-CCI injury, a unilateral craniotomy (6.0 mm) was made in an anesthetized rat centered midway between lambda and bregma. The craniotomy exposed the dura without damaging the meninges or the cortex. An 5.0 mm diameter impactor tip of an electromagnetic contusion device (MyNeurolab, St. Louis, MO) was placed into the craniotomy. The impactor tip was then removed, a plastic plate covering the craniotomy was cemented (Grip Cement, Dentsply, York, PA) to the skull and the incision sutured closed.

Rats received the same treatment for moderate CCI injury as shams except after the impactor tip was placed into the craniotomy, it compressed the cortex to a depth of 2.5 mm at 4 m/s. Both

sham- and moderately-injured rats were returned to their home cages for one week followed by testing on a hierarchy of behavioral tasks developed by the awardee (1). Day 7 post-surgery consisted of open-field, and passive avoidance. Injured and sham-injured rats performed similarly on these tasks suggesting a lack of motor deficits, and similar ability to explore a novel environment and avoid shock. On the following day, rats had six ten-minute trials to learn an active avoidance task that required avoiding a stationary shock zone on a rotating arena. The number of entrances into the shock zone was the outcome that measured active place avoidance.

The moderately injured group did not decrease the number of entrances during training since there was no significant effect of trial (Figure 1, $F_{(5,35)}=0.87$, $p > 0.5$). This suggested that the injured rats did not learn the task. In the sham-injured group, four out of 10 of the sham-injured rats reduced the number of shock-zone entrances to fewer than 5 entrances on trial 6; the remaining 6 rats had more than 10 shock zone entrances on trial 6. Thus, this sham-injured group was further subdivided into two groups (sham, <10; sham, >5) based upon the number of trial 6 shock-zone entrances.

There were significant effects of trial in both groups (sham, <5, ANOVA, $F_{(5,15)} = 5.0188$, $p<0.01$; sham, >10, $F_{(4,25)}=3.401$, $p< 0.02$) This suggests that, regardless of the number of entrances on the final trial both sham groups, acquired the task. Surprisingly, there was a significant effect of treatment among the three groups ($F_{(5,75)} = 2.848$, $p < 0.02$) suggesting that the two sham subgroups significantly differed in their ability to acquire the active avoidance task. These data further suggest that the active avoidance task has a sensitivity to detect injury that was not seen in previous studies. We are presently doing analyzing the histology of the three groups to further understand what occurs when the brain is damaged by crainiotomy. The effect of craniotomy on the CCI has been recently discussed in a recent paper (3). Interestingly, the authors described histological changes occurring following craniotomy, but could not detect any behavioral deficits. In contrast behavioral deficits could be readily seen in sham-injured animals.

Task 1 Optimization of the dosing of MINO and NAC

Dosing of MINO (45mg/kg) plus NAC (150mg/kg) to rats one hour after moderate CCI has been previously shown to synergistically improved performance on the active place task (2). Task 1 will alter the amount of both MINO and NAC separately to obtain a more complete dose-response. Preliminary data from this task is shown in figure 2.

Rats received either sham- or moderate CCI The moderately injured group did increase the number of entrances during training since there was a significant treatment effect ($F_{(3, 17)} = 4.54$ $p< 0.02$, ANOVA). There was a strong, but not yet significant trend for both MINO (45mg/kg) plus NAC (150mg/kg), and MINO (90mg/kg) plus NAC (150mg/kg) to reduce the number of entrances. The number of entrances, however, did not appear different between the two treated groups. These data repeat earlier finding that MINO plus NAC improves cognition after moderate CCI, but increasing the MINO concentration does provide a further increase in efficacy. Additional doses of MINO plus NAC will be tested to determine the optimal dosing of both drugs. Histological assessment is in progress of the various groups.

Key research accomplishments

Increasing the dose of MINO does not appear to change the efficacy of MINO plus NAC using number of entrance in the active avoidance task as a outcome.

Reportable outcomes

Dr. Bergold presented a portion of this work at the 2011 Experimental Biology Meeting at Washington DC 4/9/2011-4/13/2011 in the symposium: Traumatic Brain Injures: Anatomical Challenges and Possibilities for Repair

Dr. Bergold presented a portion of this work at the 2011 Society for Neuroscience Meeting in Washington DC 11/12/2011-11/15/2011.

Conclusions

No conclusions can yet be made.

References

1. Abdel Baki, S.G., et al., Minocycline synergizes with N-acetylcysteine and improves cognition and memory following traumatic brain injury in rats. PLoS One, 2010. 5(8): p. e12490.

2. Abdel Baki, S.G., et al., A hierarchy of neurobehavioral tasks discriminates between mild and moderate brain injury in rats. Brain Res, 2009. 1280: p. 98-106.

3. Cole, et al., Craniotomy: True Sham for Traumatic Brain Injury, or a Sham of a Sham? A. Neurotrauma 28: 359-369.

Appendix

Figures

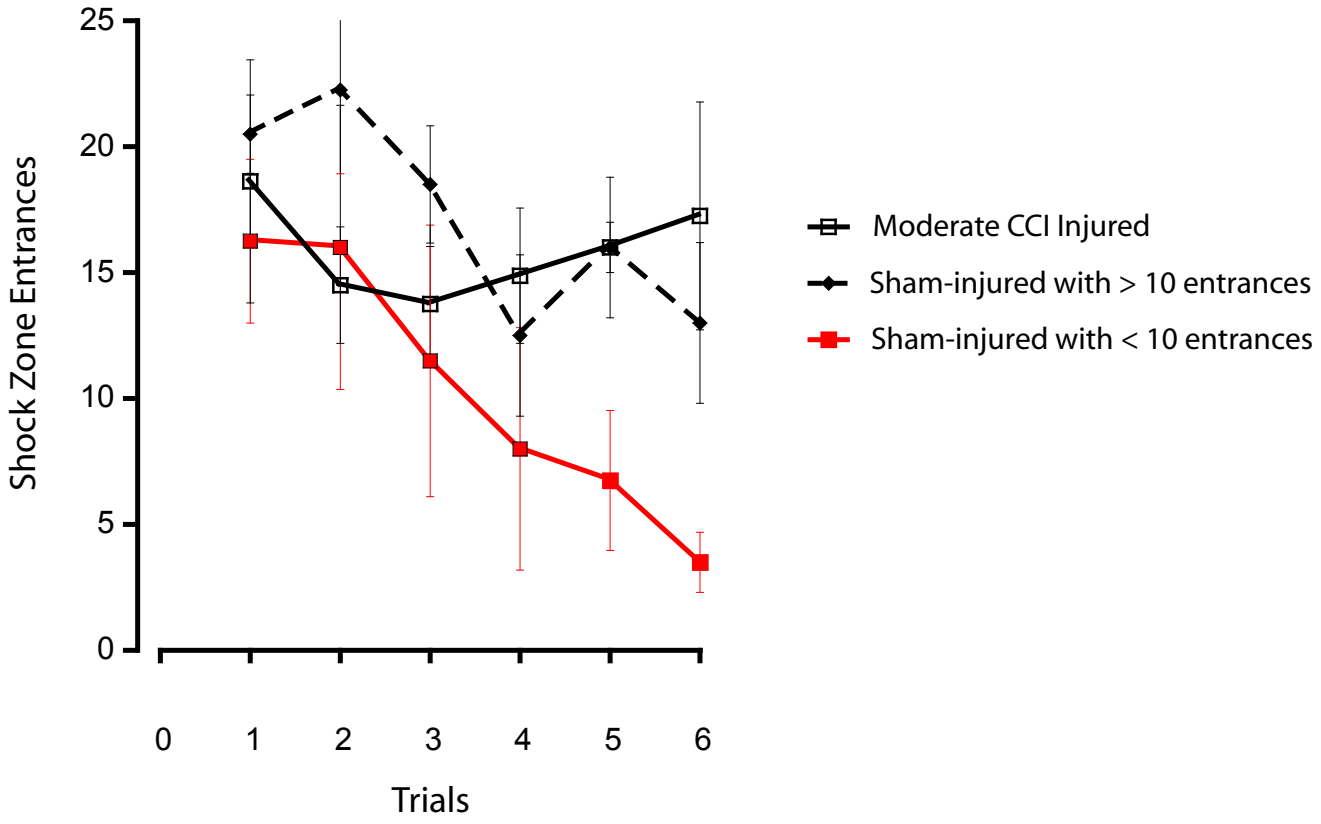

Figure 1 Two groups of sham-injured rats that have difference performance on the active avoidance task. Rats received either sham-CCI, consisting of a craniotomy; or moderate CCI. Rats were returned on their home cages for one week. They were then tested on open field and passive place avoidance. The following day the rats received six ten-minute trials of active place avoidance and the number of entrances in the shock zone assessed. The sham-injured group respond as a uniform group to the task was subdivided into two based on the number of entrances. The moderately injured group did not decrease the number of entrances during the six trials since there was no significant effect of trial ($F_{(5,35)}$=0.87, $p > 0.5$). This suggested that the injured rats could not acquire the task. In the sham-injured group, four out of 10 of the sham-injured rats reduced the number of shock-zone entrances to fewer than 5 entrances on trial 6; the remaining 6 rats had more than 10 shock zone entrances on trial 6. Thus, this sham-injured group was further subdivided into two groups (sham, >10; sham, >5) based upon the number of trial 6 shock-zone entrances. There were significant effects of trial in both groups (<10, ANOVA, $F_{(5,15)} = 5.0188$, $p<0.01$; >10, $F_{(4,25)}$=3.401, $p< 0.02$) This suggests that both sham groups could acquire the task. Surprisingly, there was a significant effect of treatment among the three groups ($F_{(5,75)} = 2.848$, $p < 0.02$) suggesting that the two sham subgroups differed in their ability to acquire the active avoidance task.

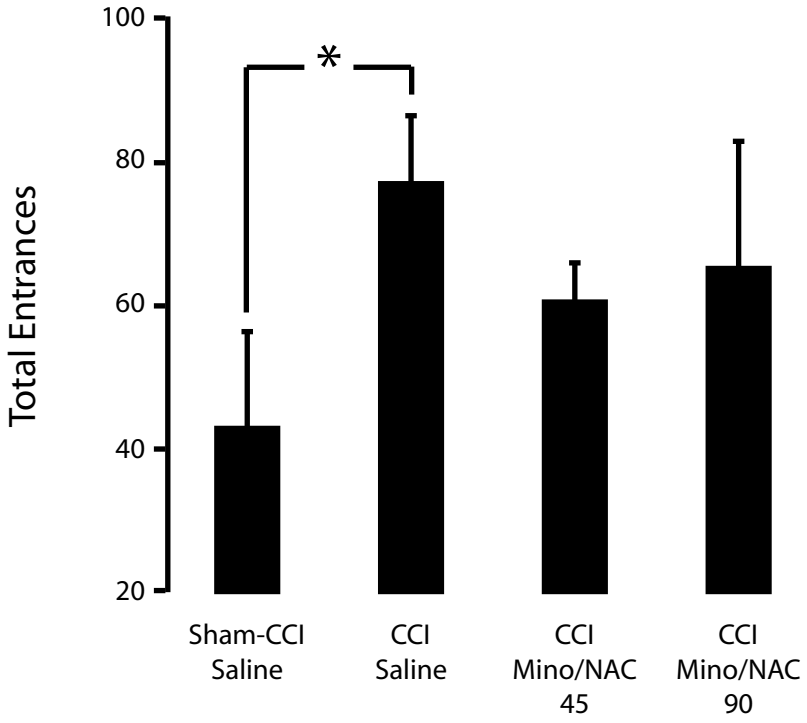

Figure 2 MINO plus NAC improves cognition after moderate CCI Four groups of rats were tested: sham-CCI, saline; moderate CCI, saline; moderate CCI, MINO (45mg/kg) NAC (150mg/kg), and moderate CCI, MINO (90mg/kg) NAC (150 mg/kg). There were four rats per group except for sham-CCI which had 5 rats. Rats received either sham- or moderate-CCI. The rats were returned to their home cages and dosed with drugs 1 hour, 1 and 2 days after surgery. Seven days after surgery, rats received 2 days of behavioral testing that included six ten-minute trials of active place avoidance. The experimental outcome was total number of entrances into the shock zone (*$p<0.01$, Student Neuman Keuls Post-test.

Abstracts of unpublished results

1) Abstract of a presentation by Dr. Bergold at the 2011 Experimental Biology Meeting in Washington DC 4/9/2011-4/13/2011 in the symposium: Traumatic Brain Injures: Anatomical Challenges and Possibilities for Repair

Evaluation of clinically relevant models of traumatic brain injury

Peter John Bergold[1], Margalit Haber[1], Raymond J. Grill[2], Sara A. Orsi[3], Pramod K. Dash[3], Andre A. Fenton[1], Samah Abdel Baki[1]. [1]Physiol. and Pharmacol., SUNY-Downstate Medical Center, Brooklyn, NY, [2]Integrative Biology and Pharmacology, [3]Physiol. and Pharmacol., U. of Texas Medical Center, Houston, TX

Despite a large need, there are presently no treatments for traumatic brain injury (TBI). This study examined how the combination of minocycline and N-acetylcysteine (MINO/NAC) synergistically

improved cognition and memory in a mild controlled cortical impact (mCCI) model of TBI. mCCI induced a long-lasting loss of axons and myelin. MINO/NAC promoted remyelination without affecting axonal loss. While MINO alone induced remyelination and provided some improvement in cognition, NAC was needed for further improvements in cognition and for restoration of long-term memory. Fingolimod, a recently approved drug to treat multiple sclerosis, appeared also to induce remyelination and partially improve cognition. The improvement of cognition by MINO or Fingolimod was less than with MINO/NAC. These studies suggest that remyelination is needed to improve cognition following mild TBI, but additional therapeutic targets are needed to restore long-term memory.

2) Abstract of a presentation by Dr. Bergold at the 2011 Society for Neuroscience Meeting in Washington DC

Minocycline and N-acetylcysteine promote remyelination after traumatic brain injury

*P. J. BERGOLD[1], M. HABER[2], N. GRINKINA[2], R. J. GRILL[3], S. A. ORSI[3], P. DASH[4], S. G. ABDEL-BAKI[2,5];
[2]Physiol. and Pharmacol., [1]SUNY-Downstate Med. Cen., BROOKLYN, NY; [3]Integrative Biol. and Pharmacol., [4]Neurobio. and Anat., Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [5]BioSignal Group, Brooklyn, NY

Abstract: Traumatic brain injury (TBI) may be better treated with drug combinations than single drugs. The combination of Minocycline (MINO, 45 mg/kg) and N-acetylcysteine (NAC, 150 mg/kg) were previously show to improve cognition and memory in rats when dosed one hour after a controlled cortical impact (CCI) that models moderate TBI (Abdel Baki, et al., PLoS One, (2010)). We now report that MINO/NAC is also effective in limiting injury when dosed one hour after rats received a mild form of CCI (mCCI). Within 2 days after mCCI, activated astrocytes and microglia are seen in corpus callosum and other white matter tracts. Within 4 days, corpus callosum lost 28.5 ± 7.9% (n=4) of axons and 74.5 ± 5.3% (n=4) of myelin content as measured by Luxol fast blue dye binding. This myelin loss remains unchanged 10 days later. mCCI also produces behavioral deficits. Injured rats are no different than sham-injured rats in avoiding a shock zone in an active avoidance task, but are completely impaired the following day when the shock zone was shifted 180°. This impairment is still evident one month after mCCI. Treatment with the drug combination of MINO and NAC beginning one hour after mCCI significantly decreases astrocytic (57.2 ± 7.0%, ANOVA, $F(3,10) = 31.58$, $p<0.0005$) and microglial activation (88.9 ± 8.0%, $F(3,10) = 11.9$, $p<0.005$). The drugs have no effect on axon loss or myelin content or the loss of myelin basic protein. These data suggest that mCCI produces a rapid white matter injury that is not effected by the drugs. Fourteen days after injury, however, myelin content in corpus callosum was significantly increased 38.8 ± 8.5% ($F(3,11) = 16.35$, $p<0.005$). The increase in myelin induced by MINO plus NAC after mCCI suggest that the drugs work by remyelination. Repair of white matter by MINO/NAC may underlie the ability of the drugs to improve cognition and memory after mCCI.